MOLECULAR AND CELLULAR BIOLOGY, Jan. 1994, p. 700–712
0270-7306/94/$04.00+0

Vol. 14, No. 1

Maf Nuclear Oncoprotein Recognizes Sequences Related to an AP-1 Site and Forms Heterodimers with both Fos and Jun

KOHSUKE KATAOKA, MAKOTO NODA, AND MAKOTO NISHIZAWA*

Department of Viral Oncology, Cancer Institute, 1-37-1 Kami-Ikebukuro, Toshima-ku, Tokyo 170 Japan

Received 6 July 1993/Returned for modification 25 August 1993/Accepted 7 October 1993

The v-*maf* oncogene, identified from AS42 avian retrovirus, encodes a nuclear bZip protein. To elucidate the molecular mechanism of cell transformation induced by this oncogene, we determined the specific binding sequences of its product. Maf protein recognized two types of relatively long palindromic consensus sequences, TGCTGACTCAGCA and TGCTGACGTCAGCA, at roughly equal efficiency. The middle parts of these Maf-binding sequences completely match with two binding sequences for AP-1 transcription factor, i.e., phorbol 12-*O*-tetradecanoate-13-acetate (TPA)-responsive element (TRE) and cyclic AMP responsive element, suggesting partial overlapping of the target genes for Maf and AP-1. Furthermore, Maf efficiently formed heterodimers with the components of AP-1, Fos and Jun, through their leucine zipper structures, and these heterodimers show binding specificities distinct from those for Maf-Maf and Jun-Jun homodimers. Thus, a multiple combination of the dimers should generate a greatly expanded repertoire of transcriptional regulatory potential. DNA data base search for the Maf-binding consensus sequences suggested that some of the TRE-like *cis* elements reported previously may actually be the targets for Maf family proteins or their heterodimers with other bZip proteins.

The *maf* oncogene, which we identified from the genome of AS42 avian transforming retrovirus (32, 56), encodes a protein containing a typical bZip structure, a motif for protein dimerization and DNA binding (36, 76). As expected from its structure, the product of this oncogene, Maf, forms homodimers through its leucine zipper structure and localizes predominantly in the nucleus (31). Structure-function studies revealed that a region of about 100 amino acid residues, which is located near its carboxyl terminus and contains an almost entire bZip motif, is essential for the basal transforming activity of v-Maf on chicken embryo fibroblasts and that the amino-terminal portion has additional enhancing effects on this activity (31). The functional importance of the bZip domain was also suggested by the structures of *maf*-related genes. We have so far isolated chicken cDNA and genomic DNA clones of four *maf*-related genes, which we named *mafK*, *mafF*, *mafB*, and *mafG* (16; unpublished results). In addition, Swaroop et al. (75) reported another distinct member of the *maf* gene family, *NRL*, which is specifically expressed in neural retina cells. The predicted amino acid sequences of the products of these *maf*-related genes are well conserved in their putative DNA-binding domain, suggesting that these proteins recognize common DNA sequences.

Interestingly, the putative DNA-binding domain of the Maf proteins also shows weak homology with the corresponding region of the members of two well-characterized transcription factor groups, the AP-1 and ATF/CREB families (56). AP-1, initially defined as a transcriptional regulatory activity, consists of proteins of the Jun and Fos families. To date, three Jun proteins (c-Jun, JunB, and JunD) and four Fos family members (c-Fos, Fra-1, FosB, and Fra-2) have been identified as components of AP-1 (7, 9, 24, 44, 54, 63, 64, 78). Among these proteins, JunB and JunD share the highest amino acid sequence identity (about 30%) with v-Maf in the putative DNA-binding domain.

These AP-1 proteins, with the exception of JunD, are known to be strongly and immediately induced by treatment with phorbol ester tumor promoters, polypeptide hormones, and other growth-stimulatory signals (reviewed in references 10 and 77). It is also well known that the AP-1 complexes recognize two *cis*-acting elements, the phorbol 12-*O*-tetradecanoate-13-acetate (TPA)-responsive element (TRE; TGAC TCA) and the cyclic AMP-responsive element (CRE; TGACGTCA). A major component of AP-1, the c-Jun protein, can bind as a homodimer to both TRE and CRE but with relatively low apparent affinity. However, in the presence of c-Fos protein, which cannot homodimerize itself, Jun preferentially forms a Jun-Fos heterodimer with increased affinity for TRE and CRE (1, 22, 33, 51, 52, 60, 65, 67, 74). The Jun-related proteins also form heterodimers with some members of another group of transcription factors, the ATF/CREB family, and preferentially bind to CRE (5, 20, 29, 40, 57). Homo- or heterodimers among the members of the ATF/CREB family also preferentially bind to CRE (21, 29, 57). From these studies, it is apparent that multiple forms of homo- and heterodimeric complexes of these bZip proteins mediate a wide variety of transcriptional regulation.

The limited but significant homology of Maf protein to AP-1 and ATF/CREB family proteins within the putative DNA-binding domain suggested the possibility that Maf protein also recognizes TRE and CRE. However, we could not detect any binding of v-Maf to the TRE of the rat aP2 gene and the CRE of the human somatostatin gene, suggesting that Maf recognizes distinct a DNA sequence(s) and *trans*-activates a different group of target genes (unpublished results).

In this study, to further explore the mechanisms of cell transformation induced by the *maf* oncogene and to delineate normal functions of *maf*-related genes, we determined consensus binding sequences of Maf. Surprisingly, the bind-

* Corresponding author. Phone: 03-3918-0111, ext. 4413. Fax: 03-5394-3816.

ing sequences of Maf were 13- or 14-base palindromic sequences which contain TRE or CRE. Furthermore, we found that Maf efficiently formed heterodimers with Jun and Fos. Binding specificities of the heterodimers, Maf/Jun and Maf/Fos, were distinct from those of the Maf homodimer and AP-1. These observations suggest that there is another layer of complexity to the network of protein-protein and protein-DNA interactions of these transcription factors that regulate the expression of wide variety of genes. A DNA data base search suggested that expression of several genes, including some growth-regulatory genes, could be modulated by Maf or its related proteins.

MATERIALS AND METHODS

Production and purification of fusion proteins. The nucleotide and amino acid residues of v-*maf* and v-*fos* genes and their products were numbered from the 5′ recombination point between the viral *gag* gene and viral oncogenes as previously described (55, 56). The bZip domain of the v-Maf protein was synthesized as a fusion protein with *Escherichia coli* maltose-binding protein (MBP), using the pMAL-c vector (New England Biolabs). The *Hae*III-*Bsu*36I fragment (nucleotide residues 720 to 1123) of the v-*maf* gene sequence, which encodes the carboxy-terminal 129 amino acid residues of v-Maf, was blunt ended with the Klenow fragment, converted to an *Eco*RI-*Xba*I fragment by the addition of linker sequences, and recloned between the *Eco*RI and *Xba*I sites of the pMal-c vector. *E. coli* cells transformed with this plasmid were cultured in rich medium and were induced to produce the recombinant protein in early log phase by addition of isopropyl-β-D-thiogalactopyranoside for 2 h. The fusion protein of the expected size (about 60 kDa) was purified to more than 95% homogeneity by amylose resin affinity chromatography as recommended by the supplier, with minor modifications. In brief, *E. coli* cells induced to produce the fusion protein were lysed by lysozyme treatment and sonication. NaCl was added to the lysate to a final concentration of 1 M, and the lysate was clarified by centrifugation and applied to the amylose resin column. To eliminate proteins and nucleic acids of *E. coli*, the column was sequentially washed with buffer A [10 mM sodium phosphate (pH 7.2), 1 mM sodium azide, 10 mM 2-mercaptoethanol, 1 mM ethylene glycol-bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid (EGTA)] containing 0.25% Tween 25 and 500 mM NaCl, buffer A containing 500 mM NaCl, buffer A containing 1 M NaCl, and buffer B (20 mM *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid [HEPES]-KOH [pH 7.9], 1 mM EDTA, 20 mM KCl, 10 mM 2-mercaptoethanol, 4 mM $MgCl_2$). Subsequently, the fusion protein was eluted with buffer B containing 10 mM maltose. The bZip domains of v-Jun and v-Fos proteins were also prepared as fusion proteins in a similar manner. To this end, the 0.65-kb *Nae*I-*Eco*RI fragment of the v-*jun* gene of avian sarcoma virus 17 (43), which encodes the carboxy-terminal 166 amino acid residues of v-Jun, and the 0.48-kb *Alw*NI-*Stu*I fragment (nucleotides 89 to 567) of the v-*fos* gene of the NK24 virus (55) were treated with T4 DNA polymerase and subcloned into the *Stu*I site of the pMAL-c vector. In the v-Fos construct, the termination codon of the fusion protein was provided by the vector sequence immediately downstream of the insert.

Selection of oligonucleotides containing Maf- and Jun-binding sites. The 64-base oligonucleotide synthesized for use in binding-site selection was 5′-TAGGCATGTAAGCTTCTC TGGG$(N)_{20}$GGGCACGTCTAGAACCTTCAAT-3′, where $(N)_{20}$ indicates a random sequence. Primers A (5′-TAGG CATGTAAGCTTCTCTGGG-3′) and B (5′-ATTGAAGGTT CTAGACGTGCCC-3′) were used for amplification of the 64-mer by polymerase chain reaction (PCR). ^{32}P-labeled double-stranded 64-mer oligonucleotides were prepared by annealing to primer B followed by treatment with DNA polymerase I Klenow fragment and polynucleotide kinase. The first cycle of selection was performed with the amylose resin affinity column, which retains either MBP-Maf or MBP-Jun. After equilibration of the column with buffer B, the double-stranded oligonucleotide was applied and washed with buffer B to eliminate nonspecifically bound DNA. The specifically bound oligonucleotides were then eluted with buffer B containing 1 M NaCl. The eluted fractions were pooled and amplified by PCR. Then the selected oligonucleotides were end labeled again, incubated with the Maf fusion protein, and subjected to gel electrophoresis. A weak shifted band was observed with the selected oligonucleotides but not with unselected oligonucleotide, suggesting significant enrichment of binding sequences. The retarded oligonucleotides were then rescued by PCR and subjected to further purification. After three successive purification cycles by the gel mobility shift-PCR techniques, the oligonucleotides were digested with *Hin*dIII and *Xba*I and subcloned into pUC to analyze its nucleotide sequences.

In vitro transcription and translation. The structure and construction of deletion mutants and point mutants of the v-*maf* gene used in this study have been described previously (31). pRAM-GEM is a subclone of the v-*maf* gene based on the pGEM-4 vector, which was used to transcribe mRNA for v-Maf (31). To produce mRNA for v-Jun, the 1-kb *Sac*II-*Eco*RI fragment of avian sarcoma virus 17 which contains all of the v-Jun-coding sequence was subcloned into pGEM-4. To construct the template plasmid for the carboxy-terminal 251 amino acid residues of v-Fos (*fos*/pGEM-4), the 0.8-kb *Not*I-*Apa*I fragment of avian NK24 virus (nucleotides 215 to 1000) (55) was treated with T4 DNA polymerase to convert it to a blunt-ended fragment and was replaced with the *Nco*I-*Eco*RI insert of the pRAM-GEM plasmid. In this construct, the *Nco*I site encodes the first methionine and the 5′ noncoding sequence is derived from the 5′ noncoding sequence of the v-*src* gene of Rous sarcoma virus, which is in good agreement with Kozak's consensus sequence for translational start sites (34). Mutated versions of these templates for Jun and Fos (*jun*L3P/pGEM-4 and *fos*L3P/pGEM-4) were generated by the method of Kunkel et al. (35). These mutated genes encode proteins in which the third leucine residues of the leucine repeats are substituted by prolines. The linearized plasmids were transcribed by T7 RNA polymerase and translated in vitro by using wheat germ extract (Promega).

Protein dimerization assay. In vitro translated proteins, labeled with [^{35}S]methionine were assayed immediately after synthesis. In vitro translated material (3 μl) was added to 800 μl of TNN buffer (Tris-HCl [pH 7.5], 150 mM NaCl, 1% Nonidet P-40) and 20 μl of amylose resin bound to about 200 ng of one of the MBP-fused bZip proteins. As a negative control, unbound amylose resin was used. After overnight mixing at 4°C, amylose resin was collected by centrifugation and washed three times with 800 μl of TNN buffer and the proteins retained on the resin were eluted for analysis by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis.

Electrophoretic mobility shift assays. The binding mixture (10 μl) contained 250 pg of the radiolabeled oligonucleotide, 20 mM HEPES-KOH (pH 7.9), 1 mM EDTA, 20 mM KCl, 5

mM dithiothreitol, 4 mM $MgCl_2$, 400 μg of poly(dI-dC) per ml, 100 μg of bovine serum albumin per ml, and 50 ng of the bacterially synthesized bZip protein or 4 μl of in vitro translated material. The resultant protein-DNA complexes and free probe were resolved through a 4% polyacrylamide gel in 1× TBE buffer (1× TBE is 89 mM Tris-borate and 2 mM EDTA [pH 8.3]) at 4°C.

RESULTS

Determination of specific binding sequences of Maf and Jun. To determine the recognition specificity of Maf, we first tried to produce Maf protein in *E. coli*. We previously observed that the expression of intact Maf protein was highly toxic to bacterial cells (31). On the other hand, it was revealed that a region of about 100 amino acid residues, located near the carboxy-terminal part of the v-Maf, which contains most of the bZip motif, is essential for its basal transforming activity (31). Amino acid sequences of this minimum essential domain are highly conserved among the members of Maf family proteins, suggesting that this domain is sufficient for its binding ability to specific DNA sequences. Then we decided to synthesize the minimum essential part of v-Maf in *E. coli* cells as a fusion protein with MBP. The fusion protein was purified to apparent homogeneity by amylose resin affinity chromatography. Double-stranded oligonucleotides containing 20 bases of random sequence were added to an amylose resin column which retains the MBP-Maf fusion protein. After extensive washing of the column at low salt concentration, the retained oligonucleotides were eluted with high-salt buffer. The partially purified oligonucleotides with potential Maf-binding sites were further purified by the gel mobility shift technique as described previously (59). After three rounds of purification, the purified oligonucleotides were cloned into a plasmid vector and sequenced. Most of the analyzed clones contained a palindromic sequence of 13 bases, TGCTGACTCAGCA, with one to five mismatches (Fig. 1). To our surprise, the consensus binding sequence contains the well-characterized *cis* element, TRE (TGAC TCA) in the middle. Four other clones, M33, M29, M124, and M21, contained a related sequence of 14 bases, TGCT GACGTCAGCA. This sequence encompasses the CRE (TGACGTCA) in the middle. The two consensus binding sequences are different only by insertion of one guanine base. In the following text, we call the 13-base sequence (TGCTGACTCAGCA) and the 14-base sequence (TGCT GACGTCAGCA) TRE-type MARE (Maf recognition element) and CRE-type MARE, respectively. Among the clones sequenced, none of the residues in the MAREs was completely conserved, suggesting that Maf recognizes these relatively long target sequences in a rather loose manner.

Jun is known to recognize both TRE and CRE, but there remains a possibility that Jun actually recognizes longer sequences (51, 65). Therefore, to clarify difference between the binding specificities of Maf and Jun, we determined the consensus binding sequences for Jun by using the same strategy. We prepared the bZip domain of v-Jun as a fusion protein in *E. coli* for determination of binding sequences. Figure 2 summarizes the oligonucleotide sequences selected by binding with the bZip domain of Jun. Most of the analyzed clones contained CRE, and only three clones contained TRE, suggesting that Jun had a higher affinity for CRE than for TRE. The most preferable sequences for Jun seemed to be a 10-base palindromic sequence, ATGACGT CAT, containing the CRE sequence. However, single bases at both termini of the optimal sequence were not strictly conserved, suggesting their weaker contribution for binding. In contrast, middle parts of all Jun-selected oligonucleotides completely matched with either the classical CRE (TGA CGTCA) or TRE (TGACTCA).

DNA-binding domain of Maf. In our previous study, we had constructed a series of deletion mutants and substitution mutants with mutations in the v-*maf* gene (31). To determine the minimum essential region of the v-Maf protein for its DNA-binding activity, we translated some of these mutant genes in vitro (Fig. 3A and B) and tested their DNA-binding activities (Fig. 3C). A nearly full-length form of Maf protein, PT, synthesized in vitro efficiently shifted the mobility of the probe (Fig. 3C, lane 2). Binding to the labeled probe was efficiently inhibited by addition of an excess amount of cold oligonucleotide which contained the recognition sequence but not by addition of mutated oligonucleotides (data not shown). Thus, it was now confirmed that Maf protein itself, but not the contaminating *E. coli* protein, binds to MAREs. Among the amino-terminal deletion mutants, ND1, ND4, and ND5 were able to bind DNA (lanes 3 to 5). The lack of function of ND5 in cell transformation might be attributed to its lack of transactivator function, as suggested by our recent study (31). Further deletion from its amino terminus (ND6) abolished its DNA-binding activity (lane 6). On the other hand, a carboxy-terminal deletion mutant, CD2, which retains most of the leucine repeat structure, was active for binding to MAREs (lane 8). CD3, which lacks the carboxy-terminal three leucine repeats and is deficient in dimer formation, was, however, no longer active (lane 9). Thus, in addition to the leucine repeat structure, a region of more than 50 amino acid residues is essential for its DNA-binding ability. Two substitution mutants, R22E, which has a substitution of a conserved arginine by glutamate in the putative DNA-binding domain, and L2PL4P, which has two substitutions of leucine residues of the zipper by proline residues and is defective in dimer formation, failed to bind DNA (lanes 10 and 12). Another substitution mutant, Q5H, which has enhanced cell-transforming activity (31), showed no apparent difference in DNA-binding ability (lane 11). Further studies are required to understand the mechanism of the potentiation of this mutant. The minimum essential region of Maf for its DNA-binding activity, indicated by a two-headed arrow at the bottom of Fig. 3A, is indistinguishable from that required for its basal transforming activity (31).

Partial overlapping sets of sequences are recognized by Maf and AP-1. To further characterize the binding specificity of Maf and Jun, we synthesized a series of oligonucleotides with one to six mutations in the consensus sequences for

FIG. 1. Determination of DNA recognition sequences for Maf. (A) Sequences of the 45 oligonucleotides selected by affinity for Maf. The random sequence core portion of the oligonucleotides is shown in capital letters, and the nonrandom arm sequences are shown partly in lowercase letters. Among the sequenced clones, four clones (M33, M29, M124, and M21) contained CRE-type MARE, and the others include TRE-type MARE. In two cases (M114/M120 and M1/M25), identical sequences were found in two independent isolates, which were probably derived from amplification of the same oligonucleotides. Numbers of mismatches from the consensus sequences and the orientation of each nucleotide are indicated on the right. (B) Nucleotide use matrix. The number of occurrences for the four nucleotides in the oligonucleotides which contain the TRE-type Maf recognition sequence are shown.

(A)

clone		mismatches from cons.	orientation
M38	ACCCCGTAAAAAAAATGCTGAcccagagaagctt	3	A
M109	TATACTCATAAAAATGCTGAcccagagaag	3	A
M41	TCATAAAAACGATGACTCAGcccag	3	A
M121	TTCATAATTCGCTGACTCATcccag	3	A
M108	TGGGCACGTGCTGACTCAGTccca	2	A
M130	ATATAAAATGCTGACTATGAccca	4	A
M20	TACCTTATGCTGACGTCGCAccc	3	A
M22	GAAGAAACGCTTACACTGCAccc	4	A
M7	AATAAATGCTAGCTAAGCAGcc	3	A
M36	CCATTGTGATGACTCAGTATcc	2	A
M114,120	AAATAATGCCAACTCAGCTTcc	3	A
M106	GGTTATGTATACTCAGCAATc	3	A
M122	CATGAAGCTGACTATGCATTc	3	A
M110	ACGAAGAAGACTCAGCATTC	3	A
M32	cAATGCTGACAATACAAAACG	4	A
M39	ccCTGCTGACTCGGCACCACTT	1	A
M45	cccTGCTGACGCAGATAGACAAA	3	A
M23	acgtgcccATTCAGCATTATGAGATGAC	3	A
M31	acgtgcccACTCAGCAACACATCCACTT	2	A
M47	AAACTATAAAACCGATGACTgggcacgtctaga	4	B
M107	TAAGGGTTATGATGATGACTgggcacgt	3	B
M112	CACGTTGTGGCATGCTGACAgggcacgt	3	B
M119	TTAATCCACGAATGCTGATTgggcacgt	3	B
M2	TAAGCGAAATATTGCTGGCGgggcacgt	4	B
M1,25	ACCTAATAATCGCTGACGCAgggcac	4	B
M6	CAGTGAAATCTGCTGACTAAgggcac	3	B
M19	TGGCGATTATGCTGACGCAGgggca	3	B
M34	TGCAATTCTGCTTACACAGCgggc	3	B
M28	TTTTATGCTGACTCAGCGggg	1	B
M117	AAAAATCTGATGACCGTGCAggg	4	B
M37	TTACTTGCTGACGCTGCTCTg	3	B
M4	CGGGAAGCTGATTCAGCACAg	2	B
M26	TAACTGCTGATCTGTCAAAT	5	B
M132	TAGTAGCTGACTGTGCAGAT	3	B
M5	TTGTGCTGACTAAGGCCTAG	3	B
M123	gTACGCTGATTCAGCATCATT	2	B
M131	ggTAAATGACTCAGCACAATTT	3	B
M3	ggAGTCTGACATTGCATTTTTG	5	B
M27	gggTAATGACTAAGCATAATTCA	3	B
Consensus	TGCTGACTCAGCA		

clone		mismatches from cons.	orientation
M33	TGAAAATATGCTGACGCTATcccag	4	A
M29	ccATCCATATGTCAGCATTTT	4	A
M124	ccGTGCTTACATCAGCAATATA	2	A
M21	gtgcccTTAGCTCAGCATTAACCCGT	5	A
Consensus	TGCTGACGTCAGCA		

(B)

A	17	12	19	5	2	9	2	1	**37**	-	5	8	**23**	1	4	**24**	7	12	12
C	3	7	7	6	-	**29**	2	2	-	**34**	5	**21**	1	-	**29**	5	18	11	10
G	8	8	6	1	**36**	-	-	**33**	2	-	6	7	7	**36**	4	7	6	9	9
T	11	12	7	**27**	1	1	**35**	3	-	5	**23**	3	8	2	2	3	8	6	8
Consensus				T	G	C	T	G	A	C	T	C	A	G	C	A			

(A)

clone		mismatches from cons.	orientation
J75	CAGGCNNNGTGATGACGTCAcccagagaagctt	1	A
J11	AGTTCCGCCAATGACGTCATccc	0	A
J57	CTGTGGAGTGGTGACGTCACccc	2	A
J58	ATATCCACTAATGACGTCATccc	0	A
J60	AGGGGGCACGGTGACGTCATccc	1	A
J73,79	GCGGCAAAGGATGACGTCATccc	0	A
J56	AACTCAGCTATGACGTCATTcc	0	A
J61	TTGTGGCCGATGACGTCATTcc	0	A
J65	TCACACACGATGACGTCATAcc	0	A
J12	TACCTCGATGACGTCATTAA	0	A
J51	ACGTGGGATGACGTCATGCT	0	A
J71	ACTCAGTATGACGTCATTCT	0	A
J16,64	GCAGCGGTGACGTCATATTT	1	A
J66,74	AAGTAGATGACGTCATTGAT	0	A
J69	AACTAATGACGTCATTTTA	0	A
J49	GATCAGTGACGTCATTGTGC	1	A
J59	CAATAATGACGTCAGAAAAC	1	A
J10	TTTGATGACGTCATGCACAG	0	A
J52	GATGATGACGTCATGTTAAA	0	A
J70	ATCGATGACGTCATGGCCC	0	A
J76	CCGATGACGTCATAGCACCT	0	A
J62,63	cTAATGACGTCATCTTCGGAT	0	A
J80	cAGATGACGTCATCCTACCTT	0	A
J78	tctagacgtgcccTATGACGTCATAATACAGTA	0	A
Consensus	ATGACGTCAT		

clone		mismatches from cons.	orientation
J14	ATCACCGTATGACTCATCCG	0	A
J77	AAGGTCTGACTCACACTGTG	2	A
J72	ggTATGACTCATCACCTGTGAA	0	B
Consensus	ATGACTCAT		

(B)

A	6	2	6	**20**	-	-	**24**	-	-	-	-	**24**	-	5	3	5
C	8	11	-	-	-	-	-	**24**	-	-	**24**	-	2	8	13	10
G	5	3	15	4	-	**24**	-	-	**24**	-	-	-	1	4	4	-
T	5	8	3	-	**24**	-	-	-	-	**24**	-	-	**21**	7	4	9
Consensus					A	T	G	A	C	G	T	C	A	T		

FIG. 2. Determination of optimal recognition sequences for v-Jun. (A) Aligned sequences of the oligonucleotides selected by association with the bacterially synthesized bZip domain of v-Jun. The randomized portions of the oligonucleotides are shown in capital letters, and the nonrandom arm sequences are shown partly in lowercase letters. Numbers of mismatches from the consensus sequences and the orientation of each nucleotide are indicated on the right. (B) Occurrences of the four nucleotides in the aligned oligonucleotides which contained CRE-type sequences.

Maf and Jun (Table 1). Using these oligonucleotides, we tested the gel mobility shift activity of the Maf and Jun proteins synthesized in *E. coli*. In addition, we produced the bZip domain of v-Fos in *E. coli* as a fusion protein with the MBP and tested its binding ability to these oligonucleotides in the presence of its essential partner, Jun (Fig. 4; Table 1). As expected from the partial similarity between the consensus sequences of Maf and Jun, some probes were shifted by both of them whereas some others were shifted by only one. For instance, oligonucleotide 11 was efficiently shifted by both Maf and Jun, whereas oligonucleotides 3 and 23 associated preferentially with Maf and Jun, respectively. The oligonucleotides which contain the optimal sequences for binding to Maf (oligonucleotides 1 and 2) were shifted by Maf at the highest efficiency, confirming the earlier results. The binding efficiencies of Maf to TRE-type and CRE-type MAREs were roughly equal. Any probe which contains one or two mismatches from the consensus sequences showed reduced binding efficiency to Maf (probes 3 to 16). In particular, substitutions in oligonucleotides 5 and 6 affected their binding activities more severely than others, suggesting unequal contribution of each nucleotide for binding. Four

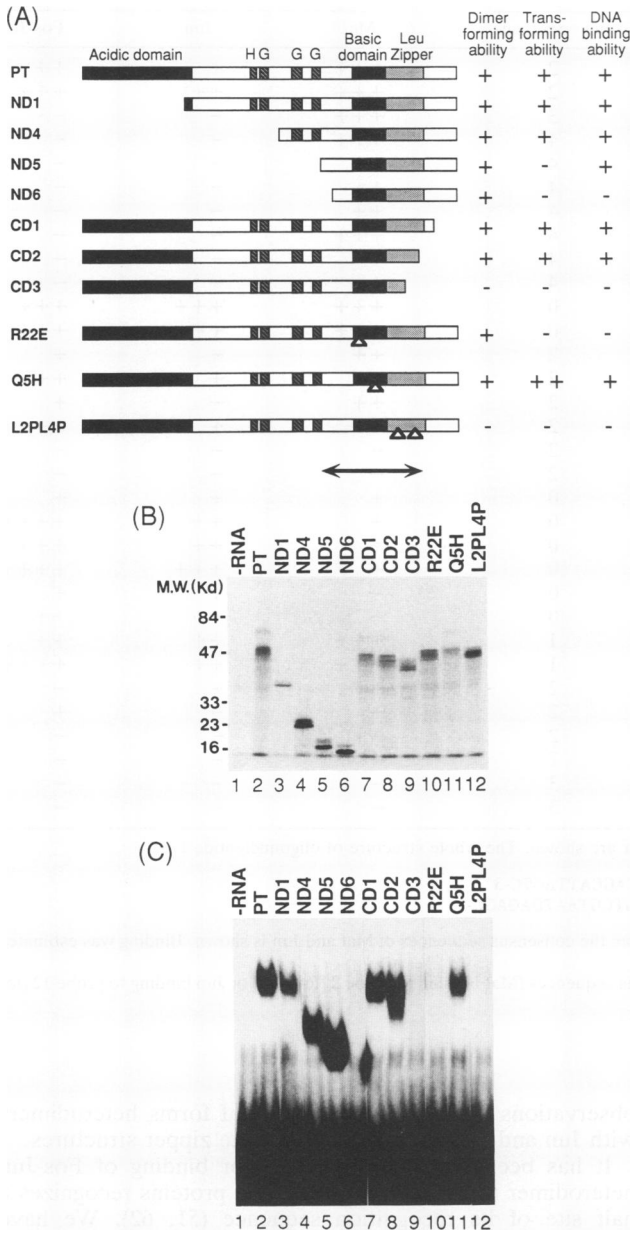

FIG. 3. DNA-binding activity of in vitro translated Maf protein. (A) Schematic representation of v-Maf mutant proteins and their DNA-binding ability. Dimer-forming and cell-transforming activities of these proteins are from Kataoka et al. (31). PT is a nearly full-length version of the v-Maf protein. Mutation sites in three substitution mutants, L2PL4P, R22E, and Q5H, are indicated by open triangles. H indicates a tract of 8 histidine residues, and G indicates three clusters of 8 to 10 glycine residues. (B) In vitro translation of v-Maf mutants. Deletion mutants or substitution mutants indicated at the top of the panel were translated in vitro in the presence of [^{35}S]methionine and analyzed by SDS-gel electrophoresis followed by fluorography. Positions of molecular size markers (in kilodaltons) are given at the left. (C) DNA-binding abilities of v-Maf mutants. Oligonucleotide 9 (Table 1) was used as a probe in this particular experiment, because of low binding activity to this nucleotide in the wheat germ extract.

oligonucleotides, which contain the optimal binding sequence for Jun homodimer (oligonucleotides 11, 12, 21, and 22), were efficiently shifted by Jun. Jun most strongly associated with the CRE-type consensus sequence (oligonucleotides 12 and 22). It is noteworthy that the binding efficiency of Jun to oligonucleotides 19 and 20, which also contain the Jun-binding consensus, was slightly lower than to oligonucleotides 12 and 13, suggesting additional sequence preference for Jun. Any mutation introduced into the TRE or CRE core sequences greatly diminished the binding to Jun (probes 3 to 10). Consistent with the previous study (65), all the oligonucleotides retarded by the Jun-Jun homodimer were also retarded by the Fos-Jun heterodimer with increased efficiencies. We could not detect any difference between the binding specificities of the Jun-Jun homodimer and the Fos-Jun heterodimer.

Heterodimer formation of Maf with Fos and Jun. We previously observed that Maf protein forms homodimers through its leucine repeat structure (31). No other cellular proteins could be detected in immunoprecipitation experiments with cell lysate prepared from AS42 virus-infected cells, suggesting that Maf acts primarily as a homodimer to transform chicken embryo fibroblasts (31, 32). However, we could not exclude the possibility that Maf associates with other bZip proteins. The similarity of the recognition sequences of Maf and Fos/Jun proteins led us to examine the ability of Maf to form heterodimers with Fos and Jun (Fig. 5). In this experiment, the MBP-fused Maf, Jun, and Fos proteins used to determine their consensus binding sequences and the ^{35}S-labeled three in vitro synthesized bZip proteins were used. The mutated proteins (Maf-L2PL4P, Jun-L3P, and Fos-L3P), which harbor one or two leucine-to-proline substitutions within their zipper structures, were included for assay as negative controls. All the proteins were synthesized efficiently (Fig. 5, lanes 1 to 6). Amylose resin bound to either one of the three MBP-fused bZip proteins was added to the lysate, and after extensive washing of the resin, proteins were recovered by boiling the resin in SDS sample buffer and were separated by SDS-polyacrylamide gel electrophoresis. In the absence of the MBP-fused bZip protein, neither of the in vitro translated proteins were efficiently precipitated by addition of control amylose resin (lanes 7 to 12). Although Jun and JunL3P proteins (lanes 9 and 10) showed weak binding to unloaded resin, much stronger and leucine zipper-dependent binding was observed with preloaded resins. For instance, when the amylose resin bound to the MBP-Maf fusion protein was used, all of the three bZip proteins were precipitated (lanes 13, 15, and 17). On the other hand, the amylose resin bound to the MBP-Maf fusion protein failed to precipitate the mutant proteins efficiently (lanes 14, 16, and 18), indicating that association of the Maf protein to Fos and Jun proteins depends on their leucine zipper motif. Consistently, in the presence of MBP-Jun or MBP-Fos fusion protein, in vitro translated Maf protein was precipitated efficiently only when the Maf protein has intact leucine zipper structure (lanes 19, 20, 25, and 26). In these conditions, also depending on the leucine zipper structure, the MBP-Jun fusion protein associated with Jun and Fos (lanes 21 to 24) and the MBP-Fos protein associated with Jun (lanes 27 and 28). These data indicate that Maf associates with both Jun and Fos, through their heptad repeat structure of leucine residues.

Binding specificities of the Maf-Jun and Maf-Fos heterodimers. To further confirm the association between Maf and the AP-1 proteins, we mixed the bacterially synthesized Maf and either Jun or Fos and subjected them to the gel

TABLE 1. Synthetic oligonucleotides used for gel mobility shift assay and evaluation of band shift data from Fig. 4

Oligonucleotide	Sequence[a]	No. of mismatches from:		Binding for[b]:		
		MAREs	Jun consensus	Maf	Jun	Fos-Jun
1	TGCTGACTCAGCA	0	2	+++	+	++
2	TGCTGACGTCAGCA	0	2	+++	++	+++
3	TGCTGATTCAGCA	1	3	+++	−	−
4	TGCTGATATCAGCA	2	4	+++	−	−
5	TGCTGGCCCAGCA	2	4	++	−	−
6	TGCTGGCGCCAGCA	2	4	++	−	−
7	TGCTTACTAAGCA	2	4	++	−	−
8	TGCTTACGTAAGCA	2	4	+++	−	−
9	TGCCGACTCGGCA	2	4	++	−	−
10	TGCCGACGTCGGCA	2	4	++	−	−
11	TGATGACTCATCA	2	0	+++	+++	+++
12	TGATGACGTCATCA	2	0	+++	+++	++++
13	TACTGACTCAGTA	2	2	++	−	+
14	TACTGACGTCAGTA	2	2	++	+	++
15	GGCTGACTCAGCC	2	2	++	−	+
16	GGCTGACGTCAGCC	2	2	++	+	+++
17	TGAGGACTCCTCA	4	2	−	−	−
18	TGAGGACGTCCTCA	4	2	−	−	−
19	TAATGACTCATTA	4	0	+	+	+
20	TAATGACGTCATTA	4	0	+	++	++++
21	GGATGACTCATCC	4	0	+	++	+++
22	GGATGACGTCATCC	4	0	+	+++	++++
23	CAATGACTCATTG	6	0	−	+	+
24	CAATGACGTCATTG	6	0	−	++	+++
25	TGCTGACTCATCA	1	1	+++	++	++
26	TGCTGACGTCATCA	1	1	+++	++	++++
27	TGCCGACTCATCC	3	2	++	−	−
28	TGCCGACGTCATCC	3	2	+	−	+
29	TGCCGACTCATTG	4	2	++	−	−
30	TGCCGACGTCATTG	4	2	+	−	−
31	TGCCGGCTCATTG	5	3	−	−	−
32	TGCCGGCGTCATTG	5	3	−	−	−

[a] Nucleotide sequences of only the middle part of the probes different each other are shown. The whole structure of oligonucleotide 1 is

5'-TCGAGCTCGGAATTGCTGACTCAGCATTACTC-3'
3'-TCGAGCCTTAACGACTGAGTCGTAATGAGAGC-5'

Nucleotide matches with the MAREs are underlined. The number of mismatches from the consensus sequences of Maf and Jun is shown. Binding was estimated by an image analyzer.

[b] Symbols: ++++, >100% of binding to the most preferable CRE-type consensus sequences (Maf binding to probe 2 [for Maf] or Jun binding to probe 12 [for Jun and Fos-Jun]); +++, 50 to 100%; ++, 20 to 50%; +, 5 to 20%; −, <5%.

mobility shift assay. Heterodimer formation between Maf and AP-1 proteins synthesized separately and subsequently mixed, however, occurred very slowly (data not shown). We then chose to cotranslate Maf and Jun or Fos in vitro and subjected them to the gel mobility shift assay with oligonucleotide probe 11 (Table 1), which can be efficiently recognized by both Maf-Maf and Jun-Jun homodimers (Fig. 6). When two Maf proteins of different lengths, PT and ND5, were cotranslated, a single additional retarded band of intermediate mobility was observed, confirming homodimer formation of Maf (Fig. 6, lane 4). When one of the two proteins was substituted with a mutant defective in dimer formation, the complex with intermediate mobility was not formed (lanes 5 and 6). Similarly, by analyzing the mixture of Maf and Jun, a band of the intermediate mobility which should be derived from the Maf-Jun heterodimer was detected, in addition to the two retarded bands of the homodimers (lane 11). Cotranslation of Maf and Fos also yielded a band with the size expected for the Maf-Fos heterodimer (lane 17). These new bands were not observed when one of the two cotranslated proteins lacked an intact leucine repeat structure (lanes 12, 13, 18, and 19). These observations again indicated that Maf forms heterodimers with Jun and Fos through their leucine zipper structures.

It has been demonstrated that, for binding of Fos-Jun heterodimer to TRE, each of the two proteins recognizes a half site of its recognition sequence (51, 62). We have established in this study that DNA-binding specificities of the Maf-Maf and Jun-Jun homodimers are clearly different. It is therefore reasonable to assume that the Maf-Jun heterodimer might most efficiently recognize asymmetrical DNA sequences consisting of half each of the two most preferable sequences of the two homodimers (Maf-Jun and Maf-Fos).

Since the AP-1 proteins are known to accumulate transiently in cells in response to growth-stimulatory signals, we examined the influence of increased amounts of AP-1 proteins on the ability of Maf to bind to specific sequences (Fig. 7). Aliquots of *maf* mRNA mixed with increasing amounts of *jun* mRNA were cotranslated for the gel mobility shift assay. With oligonucleotide probe 11, which can be efficiently recognized by both Maf and Jun, we observed a decrease in the level of Maf homodimer and an increase in the level of heterodimer (Fig. 7, lanes 2 to 5). Under these conditions,

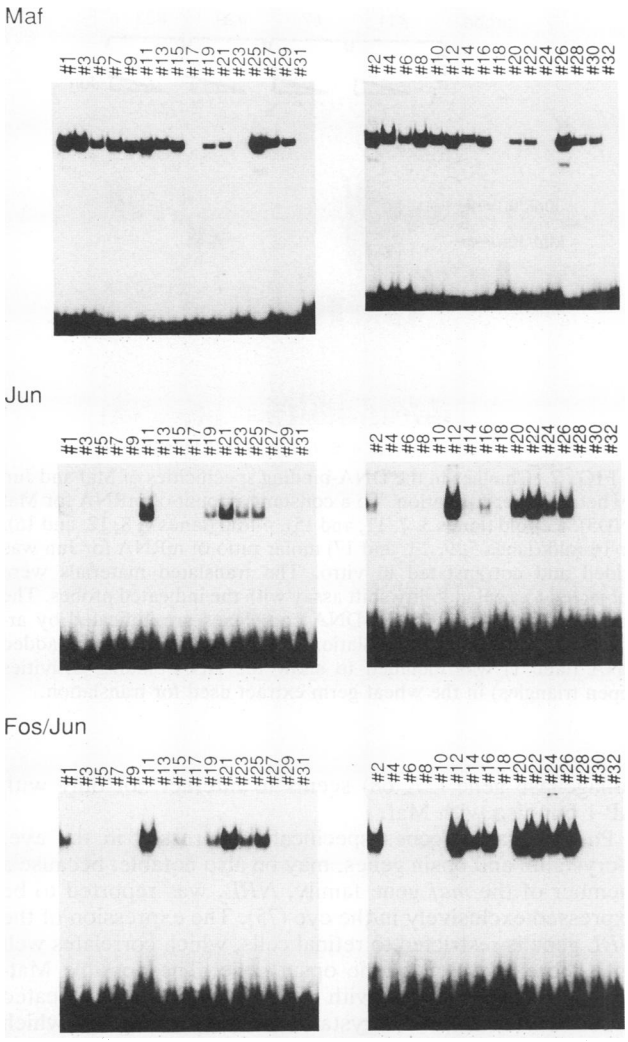

FIG. 4. Comparative analysis of DNA-binding specificities of Maf and Jun homodimers and the Fos-Jun heterodimer. The ^{32}P-labeled oligonucleotide probes indicated at the top of each panel were mixed with about 50 ng of MBP-Maf, MBP-Jun, or a mixture of MBP-Jun and MBP-Fos and were separated on polyacrylamide gels. Relative binding efficiencies of the dimers were estimated by an image analyzer and are summarized in Table 1.

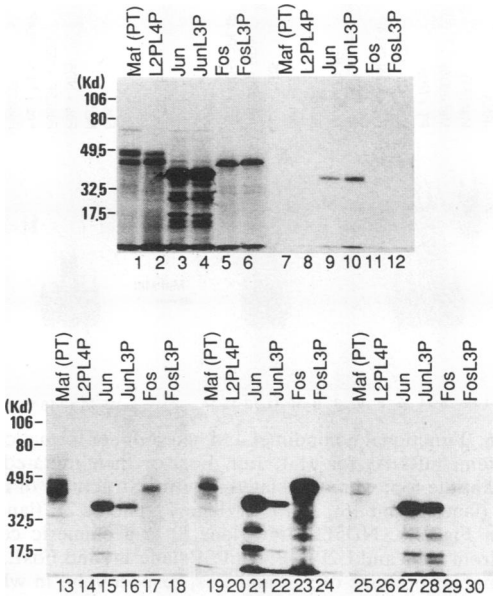

FIG. 5. Direct association of Maf and AP-1 proteins. Positions of molecular size markers (in kilodaltons) are given at the left. RNA samples as indicated at the top were translated in vitro in the presence of [^{35}S]methionine, and the products were analyzed by SDS-polyacrylamide gel electrophoresis to confirm the efficiency of translation (lanes 1 to 6). The translated proteins were mixed with amylose resin preloaded with no protein (lanes 7 to 12), MBP-Maf (lanes 13 to 18), MBP-Jun (lanes 19 to 24), or MBP-Fos (lanes 25 to 30), and the proteins retained on the resin were analyzed on SDS-polyacrylamide gels.

the binding activity of Maf to probe 7, which efficiently binds only to the Maf homodimer, was inhibited by Jun (lanes 6 to 9). In contrast, the binding activity of Maf to probe 29, which contains an asymmetrical sequence, was enhanced by the addition of Jun (lanes 10 to 13). The sequence of oligonucleotide 29 is different from the consensus sequences for Maf and Jun by four and two bases, respectively, but differs from the putative binding consensus of the Maf-Jun heterodimer (TGCTGACTCAT) by only one base. Probe 23 was efficiently recognized only by the Jun homodimer (lanes 14 to 17). It is now evident that homodimers of Maf and Jun and the Maf-Jun heterodimers have distinct target specificities. We have not detected any differences between the binding specificities of Maf-Jun and Maf-Fos heterodimers (data not shown).

Search for possible target genes in DNA data bases. We have established in this study that Maf is a sequence-specific DNA-binding protein. In our preliminary experiments, Maf has been shown to strongly activate transcription from promoters containing MARE (unpublished result), suggesting that it might be a positive transcriptional regulator. One of the next important questions would be which genes are regulated by Maf. Since Maf recognizes relatively long DNA stretches, the occurrence of such sequences in the genome was expected to be relatively less frequent. With this expectation, we tried to find possible target genes for Maf by DNA data base search. More than 20,000 segments with not more than four mismatches from MARE sequences were detected in vertebrate DNA and viral DNA sequences. Among the numerous candidates, those which are evolutionally conserved, conserved among related genes, or located within sequences reported to be functionally important for transcriptional regulation are listed in Table 2. The possible importance of these candidates is discussed below.

DISCUSSION

In the present study, we have determined consensus binding sequences for two oncogenic nuclear proteins, Maf and Jun. The binding sequences for these two proteins are identical in their middle portion but different in their flanking regions. We previously tested the binding ability of Maf to TRE and CRE but failed to detect any binding. Retrospectively, the result was reasonable because in that experiment we used the TRE of the rat aP2 gene and the CRE of the human somatostatin gene as probes for the gel mobility shift assay; these are different from TRE-type and CRE-type MAREs by 5 and 4 bases, respectively.

We have also shown that Maf, Jun, and Fos form five

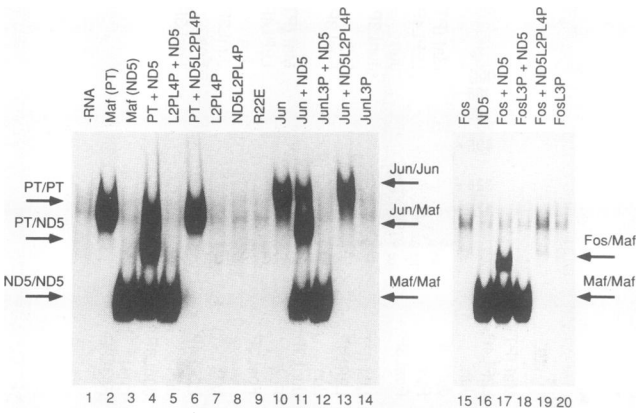

FIG. 6. Functional homodimer and heterodimer formation of the Maf protein. mRNAs for Maf, Jun, Fos, or their mutated forms, indicated at the top, were translated in vitro. Structures of PT (lane 2), ND5 (lanes 3 and 16), L2PL4P (lane 7), and R22E (lane 9) are shown in Fig. 3A. ND5L2PL4P (lane 8) is a chimeric construct derived from ND5 and L2PL4P. JunL3P (lane 14) and FosL3P (lane 20) are mutant proteins of Jun and Fos, respectively, in which the third leucine residues of the leucine repeats are substituted by prolines. The proteins were incubated with 250 pg of ^{32}P-labeled oligonucleotide 11 (Table 1) for 30 min at room temperature and were analyzed by polyacrylamide gel electrophoresis. The retarded bands corresponding to the homodimers and heterodimers are indicated by arrows.

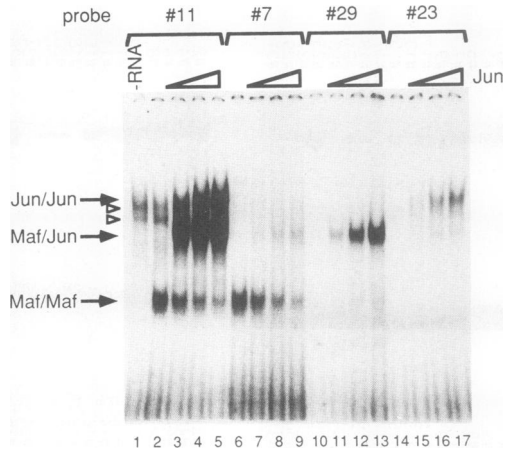

FIG. 7. Changes in the DNA-binding specificities of Maf and Jun by heterodimer formation. To a constant amount of mRNA for Maf (ND5), a 2-fold (lanes 3, 7, 11, and 15), 4-fold (lanes 4, 8, 12, and 16), or 16-fold (lanes 5, 9, 13, and 17) molar ratio of mRNA for Jun was added and cotranslated in vitro. The translated materials were subjected to a gel mobility shift assay with the indicated probes. The positions of specific protein-DNA complexes are indicated by arrows. For probe 11, the translation product prepared without added RNA (lane 1) was included to show the DNA-binding activities (open triangles) in the wheat germ extract used for translation.

types of dimers with distinct binding specificities (Fig. 8). It is also possible that Maf forms heterodimers with other bZip proteins which recognize TRE and/or CRE sequences, such as other components of AP-1 (9, 24, 44, 54, 63, 64, 78) and ATF/CREB-family proteins (14, 17, 19, 21, 25, 26, 42, 57). In addition, other members of the Maf family may participate in heterodimer formation and may lead to altered binding specificities. Potential combinations of these bZip proteins are numerous, and this may contribute to the generation of positive and negative regulatory diversity. On the basis of the DNA-binding data presented in this study, it is possible that Maf and AP-1 proteins interact with each other in either an inhibitory or a stimulatory way, depending on the target sequences. For DNA elements which bind with either of the homodimers with great preference, the presence of the heterodimeric partners will be inhibitory to the binding. For some other elements, Maf and AP-1 may act synergistically by forming heterodimers with enhanced binding affinity. With the exception of JunD (24, 63), the AP-1 components are known to be greatly and transiently induced by several types of growth-stimulatory signals. It is therefore highly likely that transient accumulation of AP-1 proteins affects the expression of target genes for Maf. Conversely, in cells which strongly express Maf, it is possible that the transcriptional stimulatory effect of AP-1 is partly modified by Maf.

As far as we have examined, expressions of c-*maf* and its related genes are not affected by mitogen treatment of the cells and are rather tissue specific (16, 56). It is therefore possible that the Maf-related proteins play important roles in tissue-specific expression of some cellular genes rather than in cell growth per se. A DNA data base search suggested that the TRE-like sequence (the E element) in the liver-specific enhancer of human hepatitis B virus (12, 72) may interact with both Maf and AP-1. Among the functional TREs of the cellular TPA-inducible genes, that of the 92-kDa type IV collagenase gene (27, 68) seems to interact not only with AP-1 but also with Maf.

Possible target genes specifically expressed in the eye, γ-crystallin and opsin genes, may be also notable, because a member of the *maf* gene family, *NRL*, was reported to be expressed exclusively in the eye (75). The expression of the *NRL* gene is restricted to retinal cells, which correlates well with the expression of the opsin gene. The possible Maf-binding sites associated with γ-crystallin genes are located within the so-called γ-crystallin conserved region, which was reported to be important for the transcription of these genes (38, 39).

The placental-type glutathione *S*-transferase gene may also be a target for Maf. Among the two enhancer elements reported by Sakai et al. (66), the one proximal to the cap site contains a TRE-type MARE with only one base mismatch from the consensus. Another possible Maf-binding site was identified in the electrophile-responsive element associated with the glutathione *S*-transferase Ya subunit gene, which is reported to be important for the basal promoter activity and induction of this gene by xenobiotics (15). Similarly, a transcriptional regulatory element, which also confers inducibility by xenobiotics to the rat NAD(P)H:quinone reductase gene, also contains a TRE-like sequence followed by the trinucleotide GCA (37). The similarity of these antioxidant response elements to TRE-type MARE suggests possible involvement of Maf family proteins in the inducible expression of these genes.

Some of the genes whose expression is specific in erythroid cells contain possible Maf-binding sites in their regulatory regions. For instance, possible Maf-binding sites are evolutionarily conserved in the enhancer elements of α- and β-globin genes. Interestingly, the conserved sequences are known to be associated with an erythroid specific transacting factor, NF-E2, and the binding specificity of this factor (2, 46, 47) seems to be similar to that of Maf. Very recently, NF-E2 was reported to be a heterodimeric complex which

TABLE 2. Possible target genes of Maf

Gene	Sequence[a]	Reference
TRE-type MARE		
Hepatitis B virus X gene enhancer[b]	1988 GGTTGCGTCAGCA	18
Feline leukemia virus LTR[b]	179 AGCTGAAACAGCA	11
Mouse mammary tumor virus LTR[b]	790 TGTTGACTCAGGA	49
Rous sarcoma virus LTR[b]	9017 CGATGAGTTAGCA	69
Human 92-kDa type IV collagenase 5′[b]	−82 CCCTGAGTCAGCA	27
Human opsin 5′[c]	134 TGCTGATTCAGCC	53
Mouse γ2-crystallin 5′[b,c]	−47 TGCCAACACAGCA	38
Rat glutathione *S*-transferase Pi 5′[b,c]	−64 TGTTGACTCAGCA	58
Mouse glutathione *S*-transferase Ya 5′[b]	−731 TGGTGACAAAGCA	15
Human NAD(P)H:quinone oxidoreductase 5′[b]	−464 AGTTGACTCAGCA	30
Mouse β-globin enhancer[b,c,d]	−9774 TGCTGAGTCATGC	48
	−9764 TGCTGAGTCATGC	
Human porphobilinogen deaminase[b]	−167 CAGTGACTCAGCA	8
Hamster histone *H3.2* 5′[b,c]	−251 TGGCGAGTCAGCC	3
Human *pim-1* 5′[c]	−1003 GGCTGAGGCAGCA	45
Human c-*erbB* upstream enhancer[b]	−1366 TTCAGAGACAGCA	41
Human c-*erbB* downstream enhancer[b]	130 TGCTGAGCCTGCA	41
Human insulin 5′[c]	137 TGCAGCCTCAGCC	4
Human IL-6 5′[c]	−283 TGCTGAGTCACTA	61
Human p53 5′[c]	−293 TCCTGACTCTGCA	6
Human plasminogen 5′[d]	−21 TGCTGAGCCAGTG	28
Human cathepsin G 5′[d]	−219 CACTGACTTAGCA	23
Human metallothionein-IG 5′[d]	−94 TGCGGACTCAGCG	13
CRE-type MARE		
Human corticotropin-releasing factor precursor gene 5′[c]	−1215 CGTTGACGTCACCA	73

[a] Positions of the sequences are indicated by the numbering system of the references. Nucleotide residues matched with the consensus sequences are underlined.
[b] Located within sequences known to be important for transcriptional regulation. LTR, long terminal repeat.
[c] Conserved in several species.
[d] Conserved in related genes.

putatively consists of two bZip proteins, p45 and p18 (2). It is noteworthy that the smaller component, p18, which has not yet been molecularly cloned, is consistent with some of the *maf* family proteins in its molecular weight. Similarly, judged from DNA-binding specificity, a nuclear factor complex interacting with the TRE-like cell cycle-dependent enhancer element of the hamster histone *H3.2* gene (50, 71), may be related to Maf.

Several growth regulatory genes may also be the target for Maf. For example, two proto-oncogenes, *pim-1* (45, 70), and c-*erbB* (41), contain possible Maf recognition sequences in their putative regulatory regions. A possible Maf-binding site, just upstream of the cap sites of insulin genes, is conserved in several species. Also, a putative MARE is conserved in the promoter regions of interleukin-6 and p53 genes in humans, rats, and mice.

Our data indicate that multiple combinations of Maf, Jun, and Fos manifest different DNA-binding specificities. An

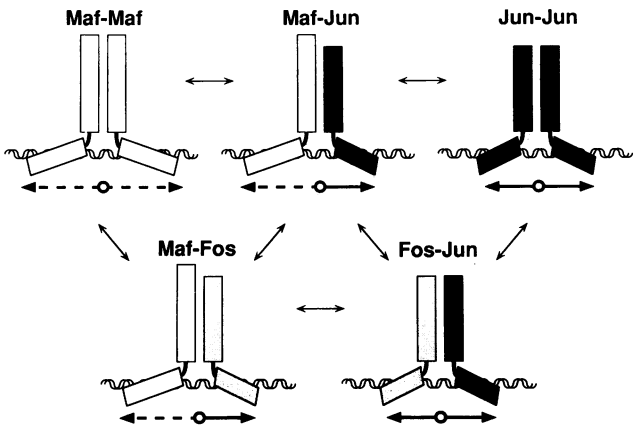

FIG. 8. Possible combinations of Maf, Jun, and Fos and their target sites. A homodimer of Maf (open rectangles) recognizes relatively long palindromic sequences (two dashed arrows) in a loose manner. The AP-1 proteins recognize shorter palindromic sequences (two solid arrows). Heterodimerization of Maf and AP-1 proteins (shaded rectangles) generates complexes capable of binding to asymmetrical hybrid DNA-recognition sites (one dashed longer arrow and one solid shorter arrow).

important implication of these findings is that different combinations of the bZip proteins may modulate distinct subsets of genes possessing the requisite DNA-binding sites. It is of interest to test the inducibility of the possible target genes in response to the overexpression of Maf, Jun, and Fos in various combinations. Such experiments may yield important insights into the normal function of, and the molecular mechanisms of cell transformation by, these three nuclear oncoproteins.

ACKNOWLEDGMENTS

We thank Shunsuke Ishii, Toshio Maekawa, Jun-ichi Fujisawa, Makoto Kinoshita, and Masayuki Yamamoto for useful suggestions. We are also grateful to Ikuko Takahashi for plasmid construction, Sharad Kumar for careful review of the manuscript, and Peter K. Vogt for providing the recombinant clone of v-*jun*.

This work was supported in part by a research grant of the Princess Takamatsu Cancer Research Fund, Uehara Memorial Foundation, and a grant-in-aid for cancer research from the Ministry of Education, Science and Culture of Japan to M. Nishizawa.

REFERENCES

1. **Abate, C., D. Luk, and T. Curran.** 1991. Transcriptional regulation by Fos and Jun in vitro: interaction among multiple activator and regulatory domains. Mol. Cell. Biol. **11**:3624–3632.
2. **Andrews, N. C., H. Erdjument-Bromage, M. B. Davidson, P. Tempst, and S. H. Orkin.** 1993. Erythroid transcription factor NF-E2 is a haemopoietic-specific basic-leucine zipper protein. Nature (London) **362**:722–728.
3. **Artishevsky, A., S. Wooden, A. Sharma, E. Resendez, and A. S. Lee.** 1987. Cell-cycle regulatory sequences in a hamster histone promoter and their interaction with cellular factors. Nature (London) **328**:823–827.
4. **Bell, G. I., R. L. Pictet, W. J. Rutter, B. Cordell, E. Tischer, and H. M. Goodman.** 1980. Sequence of the human insulin gene. Nature (London) **284**:26–32.
5. **Benbrook, D. M., and N. C. Jones.** 1990. Heterodimer formation between CREB and JUN proteins. Oncogene **5**:295–302.
6. **Bienz-Tadmor, B., R. Zakut-Houri, S. Libresco, D. Givol, and M. Oren.** 1985. The 5′ region of the p53 gene: evolutionary conservation and evidence for a negative regulatory element. EMBO J. **4**:3209–3213.
7. **Bohmann, D., T. J. Bos, A. Admon, T. Nishimura, P. K. Vogt, and R. Tjian.** 1987. Human proto-oncogene c-*jun* encodes a DNA binding protein with structural and functional properties of transcription factor AP-1. Science **238**:1386–1392.
8. **Chretien, S., A. Dubart, D. Beaupain, N. Raich, B. Grandchamp, J. Rosa, M. Goossens, and P.-H. Romeo.** 1988. Alternative transcription and splicing of the human porphobilinogen deaminase gene result either in tissue-specific or in housekeeping expression. Proc. Natl. Acad. Sci. USA **85**:6–10.
9. **Cohen, D. R., and T. Curran.** 1988. *fra*-1: a serum-inducible, cellular immediate-early gene that encodes a Fos-related antigen. Mol. Cell. Biol. **8**:2063–2069.
10. **Distel, R. J., and B. M. Spiegelman.** 1990. Protooncogene c-*fos* as a transcription factor. Adv. Cancer Res. **55**:37–55.
11. **Donahue, P. R., E. A. Hoover, G. A. Beltz, N. Riedel, V. M. Hirsch, J. Overbaugh, and J. I. Mullins.** 1988. Strong sequence conservation among horizontally transmissible, minimally pathogenic feline leukemia viruses. J. Virol. **62**:722–731.
12. **Faktor, O., S. Budlovsky, R. Ben-Levy, and Y. Shaul.** 1990. A single element within the hepatitis B virus enhancer binds multiple proteins and responds to multiple stimuli. J. Virol. **64**:1861–1863.
13. **Foster, R., N. Jahroudi, U. Varshney, and L. Gedamu.** 1988. Structure and expression of the human metallothionein-IG gene. J. Biol. Chem. **263**:11528–11535.
14. **Foulkes, N. S., E. Borrelli, and P. Sassone-Corsi.** 1991. CREM gene: use of alternative DNA-binding domains generates multiple antagonists of cAMP-induced transcription. Cell **64**:739–749.
15. **Friling, R. S., S. Bergelson, and V. Daniel.** 1992. Two adjacent AP-1-like binding sites form the electrophile-responsive element of the murine glutathione S-transferase Ya subunit gene. Proc. Natl. Acad. Sci. USA **89**:668–672.
16. **Fujiwara, K. T., K. Kataoka, and M. Nishizawa.** 1993. Two new members of the *maf* oncogene family, *mafK* and *mafF*, encode nuclear b-Zip proteins lacking putative trans-activator domain. Oncogene **8**:2371–2380.
17. **Gaire, M., B. Chatton, and C. Kedinger.** 1990. Isolation and characterization of two novel, closely related ATF cDNA clones from HeLa cells. Nucleic Acids Res. **18**:3467–3473.
18. **Galibert, F., E. Mandart, F. Fitoussi, P. Tiollais, and P. Charnay.** 1979. Nucleotide sequence of hepatitis B virus genome (subtype ayw) cloned in *E. coli*. Nature (London) **281**:646–650.
19. **Gonzalez, G. A., K. K. Yamamoto, W. H. Fischer, D. Karr, P. Menzel, W. Biggs III, W. W. Vale, and M. R. Montminy.** 1989. A cluster of phosphorylation sites on the cyclic AMP-regulated nuclear factor CREB predicted by its sequence. Nature (London) **337**:749–752.
20. **Hai, T., and T. Curran.** 1991. Cross-family dimerization of transcriptional factors Fos/Jun and ATF/CREB alters DNA binding specificity. Proc. Natl. Acad. Sci. USA **88**:3720–3724.
21. **Hai, T., F. Liu, W. J. Coukos, and M. R. Green.** 1989. Transcription factor ATF cDNA clones: an extensive family of leucine zipper proteins able to selectively form DNA-binding heterodimers. Genes Dev. **3**:2083–2090.
22. **Halazonetis, T. D., K. Georgopoulos, M. E. Greenberg, and P. Leder.** 1988. c-Jun dimerizes with itself and with c-Fos, forming complexes of different DNA binding affinities. Cell **55**:917–924.
23. **Heusel, J. W., R. D. Hanson, G. A. Silverman, and T. J. Ley.** 1991. Structure and expression of a cluster of human hematopoietic serine protease genes found on chromosome 14q11.2. J. Biol. Chem. **266**:6152–6158.
24. **Hirai, S.-I., R.-P. Ryseck, F. Mechta, R. Bravo, and M. Yaniv.** 1989. Characterization of *junD*: a new member of the *jun* proto-oncogene family. EMBO J. **8**:1433–1439.
25. **Hoeffler, J. P., T. E. Meyer, Y. Yun, J. L. Jameson, and J. F. Habener.** 1988. Cyclic AMP-responsive DNA-binding protein: structure based on a cloned placental cDNA. Science **242**:1430–1433.
26. **Hsu, J.-C., T. Laz, K. L. Mohn, and R. Taub.** 1991. Identification of LRF-1, a leucine-zipper protein that is rapidly and highly induced in regenerating liver. Proc. Natl. Acad. Sci. USA

88:3511–3515.
27. **Huhtala, P., A. Tuuttila, L. T. Chow, J. Lohi, J. Keski-Oja, and K. Tryggvason.** 1991. Complete structure of the human gene for 92-kDa type IV collagenase. J. Biol. Chem. **266:**16485–16490.
28. **Ichinose, A.** 1992. Multiple members of the plasminogen-apolipoprotein(a) gene family associated with thrombosis. Biochemistry **31:**3113–3118.
29. **Ivashkiv, L. B., H.-C. Liou, C. J. Kara, W. W. Lamph, I. M. Verma, and L. H. Glimcher.** 1990. mXBP/CRE-BP2 and c-Jun form a complex which binds to the cyclic AMP, but not to the 12-*O*-tetradecanoylphorbol-13-acetate, response element. Mol. Cell. Biol. **10:**1609–1621.
30. **Jaiswal, A. K.** 1991. Human NAD(P)H:quinone oxidoreductase (NQO_1) gene structure and induction by dioxine. Biochemistry **30:**10647–10653.
31. **Kataoka, K., M. Nishizawa, and S. Kawai.** 1993. Structure-function analysis of the *maf* oncogene product, a member of the b-Zip protein family. J. Virol. **67:**2133–2141.
32. **Kawai, S., N. Goto, K. Kataoka, T. Saegusa, H. Shinno-Kohno, and M. Nishizawa.** 1992. Isolation of the avian transforming retrovirus, AS42, carrying the v-*maf* oncogene and initial characterization of its gene product. Virology **188:**778–784.
33. **Kouzarides, T., and E. Ziff.** 1988. The role of the leucine zipper in the fos-jun interaction. Nature (London) **336:**646–651.
34. **Kozak, M.** 1986. Point mutations define a sequence flanking the AUG initiator codon that modulates translation by eukaryotic ribosomes. Cell **44:**283–292.
35. **Kunkel, T. A., J. D. Roberts, and R. A. Zakour.** 1987. Rapid and efficient site-specific mutagenesis without phenotypic selection. Methods Enzymol. **154:**367–382.
36. **Landschulz, W. H., P. F. Johnson, and S. L. McKnight.** 1988. The leucine zipper: a hypothetical structure common to a new class of DNA binding proteins. Science **240:**1759–1764.
37. **Li, Y., and A. K. Jaiswal.** 1992. Regulation of human NAD(P)H: quinone oxidoreductase gene. J. Biol. Chem. **267:**15097–15104.
38. **Lok, S., M. L. Breitman, A. B. Chepelinsky, J. Piatigorsky, R. J. M. Gold, and L. C. Tsui.** 1985. Lens-specific promoter activity of a mouse γ-crystallin gene. Mol. Cell. Biol. **5:**2221–2230.
39. **Lok, S., W. Stevens, M. L. Breitman, and L.-C. Tsui.** 1989. Multiple regulatory elements of the murine γ2-crystallin promoter. Nucleic Acids Res. **17:**3563–3582.
40. **Macgregor, P. F., C. Abate, and T. Curran.** 1990. Direct cloning of leucine zipper proteins: Jun binds cooperatively to the CRE with CRE-BP1. Oncogene **5:**451–458.
41. **Maekawa, T., F. Imamoto, G. T. Merlino, I. Pastan, and S. Ishii.** 1989. Cooperative function of two separate enhancers of the human epidermal growth factor receptor proto-oncogene. J. Biol. Chem. **264:**5488–5494.
42. **Maekawa, T., H. Sakura, C. Kanei-Ishii, T. Sudo, T. Yoshimura, J. Fujisawa, M. Yoshida, and S. Ishii.** 1989. Leucine zipper structure of the protein CRE-BP1 binding to the cyclic AMP response element in brain. EMBO J. **8:**2023–2028.
43. **Maki, Y., T. J. Bos, C. Davis, M. Starbuck, and P. K. Vogt.** 1987. Avian sarcoma virus 17 carries the *jun* oncogene. Proc. Natl. Acad. Sci. USA **84:**2848–2852.
44. **Matsui, M., M. Tokuhara, Y. Konuma, N. Nomura, and R. Ishizaki.** 1990. Isolation of human *fos*-related genes and their expression during monocyte-macrophage differentiation. Oncogene **5:**249–255.
45. **Meeker, T. C., J. Loeb, M. Ayres, and W. Sellers.** 1990. The human *pim-1* gene is selectively transcribed in different hematolymphoid cell lines in spite of a G+C-rich housekeeping promoter. Mol. Cell. Biol. **10:**1680–1688.
46. **Mignotte, V., J. F. Eleouet, N. Raich, and P.-H. Romeo.** 1989. Cis- and trans-acting elements involved in the regulation of the erythroid promoter of the human porphobilinogen deaminase gene. Proc. Natl. Acad. Sci. USA **86:**6548–6552.
47. **Mignotte, V., L. Wall, E. deBoer, F. Grosveld, and P.-H. Romeo.** 1989. Two tissue-specific factors bind the erythroid promoter of the human porphobilinogen deaminase gene. Nucleic Acids Res. **17:**37–54.
48. **Moon, A. M., and T. J. Ley.** 1990. Conservation of the primary structure, organization, and function of the human and mouse β-globin locus-activating regions. Proc. Natl. Acad. Sci. USA **87:**7693–7697.
49. **Moore, R., M. Dixon, R. Smith, G. Peters, and C. Dickson.** 1987. Complete nucleotide sequence of a milk-transmitted mouse mammary tumor virus: two frameshift suppression events are required for translation of *gag* and *pol*. J. Virol. **61:**480–490.
50. **Naeve, G. S., A. Sharma, and A. S. Lee.** 1992. Identification of a 10-base pair protein binding site in the promoter of the hamster *H3.2* gene required for the S phase dependent increase in transcription and its interaction with a Jun-like nuclear factor. Cell Growth Differ. **3:**919–928.
51. **Nakabeppu, Y., and D. Nathans.** 1989. The basic region of Fos mediates specific DNA binding. EMBO J. **8:**3833–3841.
52. **Nakabeppu, Y., K. Ryder, and D. Nathans.** 1988. DNA binding activities of three murine Jun proteins: stimulation by Fos. Cell **55:**907–915.
53. **Nathans, J., and D. S. Hogness.** 1984. Isolation and nucleotide sequence of the gene encoding human rhodopsin. Proc. Natl. Acad. Sci. USA **81:**4851–4855.
54. **Nishina, H., H. Sato, T. Suzuki, M. Sato, and H. Iba.** 1990. Isolation and characterization of *fra-2*, an additional member of the *fos* gene family. Proc. Natl. Acad. Sci. USA **87:**3691–3623.
55. **Nishizawa, M., N. Goto, and S. Kawai.** 1987. An avian transforming retrovirus isolated from a nephroblastoma that carries the *fos* gene as the oncogene. J. Virol. **61:**3733–3740.
56. **Nishizawa, M., K. Kataoka, N. Goto, K. T. Fujiwara, and S. Kawai.** 1989. v-*maf*, a viral oncogene that encodes a "leucine zipper" motif. Proc. Natl. Acad. Sci. USA **86:**7711–7715.
57. **Nomura, N., Y.-L. Zu, T. Maekawa, S. Tabata, T. Akiyama, and S. Ishii.** 1993. Isolation and characterization of a novel member of the gene family encoding the cAMP response element-binding protein CRE-BP1. J. Biol. Chem. **268:**4259–4266.
58. **Okuda, A., M. Sakai, and M. Muramatsu.** 1987. The structure of the rat glutathione S-transferase gene and related pseudogene. J. Biol. Chem. **262:**3858–3863.
59. **Perkins, A. S., R. Fishel, N. A. Jenkins, and N. G. Copeland.** 1991. *Evi-1*, a murine zinc finger proto-oncogene, encodes a sequence-specific DNA-binding protein. Mol. Cell. Biol. **11:**2665–2674.
60. **Rauscher, F. J., III, D. R. Cohen, T. Curran, T. J. Bos, P. K. Vogt, D. Bohmann, R. Tjian, and B. R. Franza, Jr.** 1988. Fos-associated protein p39 is the product of the jun proto-oncogene. Science **240:**1010–1016.
61. **Ray, A., S. B. Tatter, L. T. May, and P. B. Sehgal.** 1988. Activation of the human "β_2-interferon/hepatocyte-stimulating factor/interleukin 6" promoter by cytokines, viruses, and second messenger agonist. Proc. Natl. Acad. Sci. USA **85:**6701–6705.
62. **Risse, G., K. Jooss, M. Neuberg, H.-J. Brüller, and R. Müller.** 1989. Asymmetrical recognition of the palindromic AP1 binding site (TRE) by Fos protein complexes. EMBO J. **8:**3825–3832.
63. **Ryder, K., A. Lanahan, E. Perez-Albuerne, and D. Nathans.** 1989. *Jun-D*: a third member of the *Jun* gene family. Proc. Natl. Acad. Sci. USA **86:**1500–1503.
64. **Ryder, K., L. F. Lau, and D. Nathans.** 1988. A gene activated by growth factors is related to the oncogene v-*jun*. Proc. Natl. Acad. Sci. USA **85:**1487–1491.
65. **Ryseck, R.-P., and R. Bravo.** 1991. c-Jun, JunB, and JunD, differ in their binding affinities to AP-1 and CRE consensus sequences: effect of Fos proteins. Oncogene **6:**533–542.
66. **Sakai, M., A. Okuda, and M. Muramatsu.** 1988. Multiple regulatory elements and phorbol 12-*O*-tetradecanoate 13-acetate responsiveness of the rat placental glutathione transferase gene. Proc. Natl. Acad. Sci. USA **85:**9456–9460.
67. **Sassone-Corsi, P., L. J. Ransone, W. W. Lamph, and I. M. Verma.** 1988. Direct interaction between fos and jun nuclear oncoproteins: role of the 'leucine zipper' domain. Nature (London) **336:**692–695.
68. **Sato, H., and M. Seiki.** 1993. Regulatory mechanism of 92 kDa type IV collagenase gene expression which is associated with invasiveness of tumor cells. Oncogene **8:**395–405.
69. **Schwartz, D. E., R. Tizard, and W. Gilbert.** 1983. Nucleotide

sequence of Rous sarcoma virus. Cell **32:**853–869.

70. **Selten, G., H. T. Cuypers, W. Boelens, E. Robanus-Maandag, J. Verbeek, J. Domen, C. van Beveren, and A. Berns.** 1986. The primary structure of the putative oncogene *pim-1* shows extensive homology with protein kinases. Cell **46:**603–611.
71. **Sharma, A., T. J. Bos, A. Pekkala-Flagan, P. K. Vogt, and A. S. Lee.** 1989. Interaction of cellular factors related to the Jun oncoprotein with the promoter of a replication-dependent hamster histone H3.2 gene. Proc. Natl. Acad. Sci. USA **86:**491–495.
72. **Shaul, Y., and R. Ben-Levy.** 1987. Multiple nuclear proteins in liver cells are bound to hepatitis B virus enhancer element and its upstream sequences. EMBO J. **6:**1913–1920.
73. **Shibahara, S., Y. Morimoto, Y. Furutani, M. Notake, H. Takahashi, S. Shimizu, S. Horikawa, and S. Numa.** 1983. Isolation and sequence analysis of the human corticotropin releasing factor precursor gene. EMBO J. **2:**775–779.
74. **Smeal, T., P. Angel, J. Meek, and M. Karin.** 1989. Different requirements for formation of Jun:Jun and Jun:Fos complexes. Genes Dev. **3:**2091–2100.
75. **Swaroop, A., J. Xu, H. Pawar, A. Jackson, C. Scolnick, and N. Agarwal.** 1992. A conserved retina-specific gene encodes a basic motif/leucine zipper protein. Proc. Natl. Acad. Sci. USA **89:**266–270.
76. **Vinson, C. R., P. B. Sigler, and S. L. McKnight.** 1989. Scissors-grip model for DNA recognition by a family of Leucine zipper proteins. Science **246:**911–916.
77. **Vogt, P. K., and T. J. Bos.** 1990. jun: oncogene and transcription factor. Adv. Cancer Res. **55:**1–35.
78. **Zerial, M., L. Toschi, R.-P. Ryseck, M. Schuermann, R. Müller, and R. Bravo.** 1989. The product of a novel growth factor activated gene, *fos B*, interacts with JUN proteins enhancing their DNA binding activity. EMBO J. **8:**805–813.